Effect of Enzymatic Modification on Chicken Surimi

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Abstract

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The impact of the addition and duration of protein modification with transglutaminase preparation of microbial origin (MTG) of a chicken surimi on its texture, rheological characteristics, water molecular dynamics, and water activity were assessed. Surimi samples were supplemented with 0.3 g/100 g MTG and incubated maximally for about 7 h at the temperature of 15°C. The greatest changes of the chicken surimi properties were observed during the initial period, i.e. during 3 h of proteins incubation with MTG. Surimi modification with transglutaminase increased the equilibrium water activity in comparison with the value obtained for the control sample. At the same time, the mean diffusion coefficient in the enzymatically modified poultry surimi sample reached the value nearly twice higher than in the control sample.

Keywords: transglutaminase; texture; rheology; NMR; water activity

Myofibrilar proteins are the most valuable protein fraction found in the mechanically recovered protein meat (MRPM). MRPMs find their technological application, primarily, as additives in the production of cheap meat commodities of low shelf-life subjected to thermal treatment. The application range of myofibrilar protein isolates (synonym: poultry surimi) is expanding into possibilities of chicken breast muscles substitution and utilisation as a full-value binding agent in restructured products or as an additive to more expensive and more stable meat articles. The final quality of gels obtained from rinsed MRPM is not always identical and depends considerably on the raw material and recovery parameters, e.g. number of rinses, kind of the rinsing agent, MRPM:water ratio etc. (Stangierski & Kijowski 2000). Technological properties of food raw materials are managed more and more frequently with the assistance of methods based on enzymatic modifications and, in the case of protein functional properties, transglutaminase (EC 2.3.2.13) (TG) is particularly promising. In the meat industry, protein crosslinking with the assistance of TG – both endogenous, occurring especially in the meat of fish, as well as that added on purpose in the course of processing activities – found practical application (Murphy *et al*. 2004; Serrano *et al*. 2004; Colmenero & Carballo 2005; Perlo *et al*. 2006). Crosslinking isopeptide bonds of ε-(γ-Glu)-Lys type, which develop under the influence of TG, can bind two different proteins or peptides as well as develop bonds inside one protein molecule. Proteins possess numerous peptide bonds, chain branchings as well as many active amino acid residues, usually situated outside the molecule and, therefore, they are sensitive to various kinds of enzymatic modification. The differences observed in protein sensitivity to TG

action depend, primarily, on the glutamine content and its availability to the enzyme but also on protein conformation (Dondero *et al*. 2006).

In order to achieve progress in understanding the functional properties of enzymatically modified poultry surimi, it is necessary to employ such physical methods and techniques which are sufficiently sensitive to the changes in intermolecular interactions taking place in the examined systems. One of such methods is basic rheology. Linear viscoelastic properties are related to physical interactions between polymer molecules of the examined system. Despite commonly applied rheometric techniques, the publications devoted to "surimi" type protein preparations obtained from poultry meat are scarce. Most of the available papers on the subject deal with proteins obtained from saltwater fish (Uresti *et al*. 2004).

Another technique employed to assess the interactions occurring between the molecules of the examined system is the nuclear magnetic resonance (NMR) method, in particular the so called relaxation in low magnetic fields which consists in the analysis of the disappearance of the NMR signal. The intensity of the signal is proportional to the number of nuclei present in the system. The velocity of the signal disappearance reflects molecular mobility in the investigated system (microdynamics) (Bertram *et al*. 2004). The process of recovery from the state of excitation to the state of equilibrium is referred to as the spin-lattice relaxation process, described by the T_1 time. The spin-spin relaxation time T_2 reflects the interactions of neighbouring spins, even in the same macromolecule.

Enzymatic modification of the protein poultry surimi leads to considerable protein conformation transformations and, together with them, to changes in the degree of the system free water binding. Naturally, this finds its reflection in changes of protein functional traits. That is why the aim of the performed experiments was to investigate the impact of transglutaminase enzyme on the texture and rheological properties as well as on the water molecular dynamics in the myofibrilar preparation obtained from MRPM.

MATERIAL AND METHODS

The basic experimental material was mechanically recovered poultry meat from broiler carcasses with removed breast muscles and legs obtained with the assistance of a French-made device Lima type RM 500 (Lima S.A.S., Quimper, France). The direct experimental material was myofibrilar preparation (MP) (synonym – chicken surimi) manufactured from MRPM. The method of obtaining the preparation was adopted from the procedure described in a patent claim (Kijowski *et al*. 1996). The meat raw material was rinsed with 0.169 mol/l aqueous solution of NaCl followed by water (MRPM:water 1:3, w/v) and next fat and connective tissue were separated with the assistance of sieves. The obtained preparation of myofibrilar proteins was divided into two parts. The control sample was taken from the first batch of the raw material, while the second batch was supplemented with 0.3 g/100 g transglutaminase preparation and incubated at 15°C for the period of 6 or 7 hours. The differences in the protein modification time resulted from the analytical specificity of the research methods applied.

The MP enzymatic modification was performed with the assistance of a preparation of commercial name ACTIVA WM of the Ajinomoto Co. Ltd. (Barentz, Poland). The applied preparation contained 1% transglutaminase of microbiological origin (MTG) (*Streptoverticillium* sp.) of 100 units/g activity and 99% maltodextrine used as the enzyme carrier (Ajinomoto's specifications). One unit was the amount of the enzyme which catalysed the formation of 1 μmol of hydroxamic acid/min at 37°C.

Determination of basic chemical composition of MP basic constituents was carried out with the assistance of standard methods, i.e. water (PN-ISO 1442:2000), protein content according to the Kjeldahl procedure (N × 6.25) (PN-A-04018: 1975/Az3:2002), fat (Soxhlet method) (PN-ISO 1444:2000) and ash (PN-ISO 936:2000) contents.

Texture measurements of the MP were performed with the assistance of a device known as Texture Analyser TA-XT2i (Texture Technologies Corp., Surrey, UK) utilising, for this purpose, the method of back extrusion using an attachment of A/BE type. The sample (approximately 80 ± 5 g) placed inside a cylinder of 50 mm inner diameter was subjected to compression by a disk of 40 mm diameter to the depth of 30 mm. The smaller disk diameter caused that part of the sample preparation was forced between the walls of the cylinder and the disk. The behaviour of the sample preparation during compression as well as during the disk return motion was analysed. The following parameters were determined on the basis of the

performed investigations: firmness (N), consistence (N·s), cohesiveness (N) and index of viscosity (N·s).

Determination of rheological properties using the DMA (Dynamic Mechanical Analysis). In the described investigations, the authors employed the method of oscillation rheology using a Dynamic-Mechanical Rheologic Analyser DMWT (Cobrabid, Poznań, Poland) which operates on the principle of free vibration analysis in the inverted torsional pendulum (Rezler & Poliszko 2010). The employed measuring system employed coaxial cylinders with the examined samples formed between them. The following rheological parameters were determined: modulus of elasticity (G_1), loss modulus (G_2) and loss tangent (tgδ). The frequency of the system own vibrations amounted to 0.4 Hz.

Determination of dynamics of water molecules using NMR. Pulse sequence λ–τ–λ/2 was used to determine the relaxation time T_1 (spin-lattice) while CPMG pulse train was used to determine the relaxation time T_2 (spin-spin). The measurements were taken using a NMR Pulse Spectrometer PS 15T by ElLab (Poznań, Poland) operating at 15 MHz.

Measurements of water activity equilibrium were conducted at the temperature of 15°C employing the analyser of water diffusion and activity ADA-7 (COBRABID, Poznań, Poland) with a system of automatic time recording of water evacuation runs from individual samples. Once the measurement temperature was reached, it was determined with 0.05°C accuracy.

Statistical analysis. In order to compare the significance of differences between the mean values, statistical verification was performed using Duncan's test for this purpose and NIR or Kruskal-Walis test for non-parametrical data. Depending on the needs, single- (ANOVA) and multi-factorial (MANOVA) analyseis of correlation were employed. To verify the assumed research hypothesis, $P \leq 0.05$ significance level of inference was adopted. The statistical analysis was carried out with the assistance of the STATISTICA PL v. 8.0 software (StatSoft Polska, Krakow, Poland).

RESULTS AND DISCUSSION

Basic composition

No statistically significant impact was observed of the supplementation with the enzymatic preparation on the chemical composition of the protein

Table 1. Texture parameters observed for the control system of myofibrilar preparation (MP) and transglutaminase modified proteins (MP + MPTG)

Incubation time (h)	Firmness (N)	Consistency (N·s)	Cohesiveness (N)	Index of viscosity (N·s)
MP				
0	$14.6^{g} \pm 0.2$	$112.3^{j} \pm 0.3$	$-10.1^{cd} \pm 0.4$	$43.2^{g} \pm 0.2$
1	$14.4^{gh} \pm 0.3$	$115.8^{f} \pm 0.2$	$-9.5^{cd} \pm 0.5$	$42.5^{h} \pm 0.3$
2	$14.7^{fg} \pm 0.3$	$114.1^{h} \pm 0.2$	$-8.9^{d} \pm 0.8$	$42.1^{h} \pm 0.2$
3	$14.7^{fg} \pm 0.3$	$116.8^{e} \pm 0.5$	$-10.1^{cd} \pm 0.6$	$39.4^{i} \pm 0.3$
4	$14.0^{hi} \pm 0.3$	$115.1^{g} \pm 0.3$	$-9.3^{d} \pm 0.4$	$39.9^{i} \pm 0.3$
5	$13.8^{hi} \pm 0.2$	$113.3^{i} \pm 0.3$	$-9.9^{cd} \pm 0.5$	$38.7^{j} \pm 0.2$
6	$13.7^{i} \pm 0.2$	$114.5^{gh} \pm 0.4$	$-10.2^{cd} \pm 0.5$	$39.5^{i} \pm 0.3$
MP + MPTG				
0	$14.2^{h} \pm 0.3$	$110.6^{l} \pm 0.3$	$-9.7^{cd} \pm 0.6$	$43.3^{g} \pm 0.3$
1	$15.1^{f} \pm 0.3$	$111.7^{k} \pm 0.2$	$-9.8^{d} \pm 0.2$	$55.3^{f} \pm 0.5$
2	$16.5^{e} \pm 0.4$	$116.1^{ef} \pm 0.4$	$-9.9^{cd} \pm 0.5$	$61.7^{e} \pm 0.3$
3	$19.2^{d} \pm 0.2$	$121.6^{d} \pm 0.3$	$-10.6^{bc} \pm 0.5$	$62.9^{d} \pm 0.4$
4	$20.4^{c} \pm 0.2$	$127.5^{bc} \pm 0.4$	$-11.9^{b} \pm 0.4$	$64.1^{c} \pm 0.4$
5	$21.3^{b} \pm 0.3$	$128.9^{b} \pm 1.0$	$-13.4^{a} \pm 0.6$	$66.5^{b} \pm 0.4$
6	$22.6^{a} \pm 0.2$	$133.2^{a} \pm 0.7$	$-14.2^{a} \pm 0.4$	$67.7^{a} \pm 0.3$

Different letters in columns denote a significant difference for means at $P \leq 0.05$ ($n = 6$; ± standard deviation)

preparation obtained as a result of MRPM rinsing. The samples obtained were characterised by the following mean basic chemical composition: 84.3 ± 0.5% water, 14.1 ± 0.3% protein, 0.8 ± 0.2% fat and 0.5 ± 0.1% ash.

Texture

The values of the texture parameters determined for MP samples with and without the supplementation with transglutaminase are shown in Table 1. In the case of the control sample, relatively small value changes of the parameters characterising the texture of protein preparations together with the incubation time prolongation were observed. The values of the texture parameters determined for MP containing microbiological preparation of transglutaminase (MPTG) were considerably higher than those for the control samples which can be noticed already in the third period of measurements. Worth noticing is a very dynamic value increase of the texture parameters occurring during 3 h of modification. The highest numerical values of the obtained factors were determined after 6 h of incubation of the preparation with the enzyme. In the analysed incubation period, the values of MP texture parameters, i.e. firmness and elasticity coefficients, increased by nearly 60%.

Low TG activity in the meat of poultry, swine, and cattle was accompanied by its slow crosslinking during sedimentation and small gel elasticity. Perhaps this also explains the poor aptitude of poultry myosin heavy chain (MHC) for crosslinking under the influence of the enzyme. The advantageous effect on the texture of rinsed but not heated bovine (Serrano *et al*. 2004) and poultry meats as well as swine and sheep meats was also observed by other researchers (Carballo *et al*. 2006).

Rheological properties

Figure 1 presents the character of the changes of the dynamic-mechanical protein preparation properties as well as of the samples supplemented with transglutaminase taking place in the course of their modification illustrated by courses of $G_1(t)$ and $G_2(t)$ dependencies. The examined protein systems show a rapid increase of both modulus of elasticity (G_1) and loss modulus (G_2) values during the initial incubation time (3–5 h). If the period of time is longer, the changes in the values of the above-mentioned moduli occur very slowly and aim asymptotically at achieving a constant value. The kinetics of the observed crosslinking is subject to the kinetics equation of the first order reaction. Therefore, the component changes of the combined modulus of elasticity(G_1) and loss (G_2) can be described by the following equation:

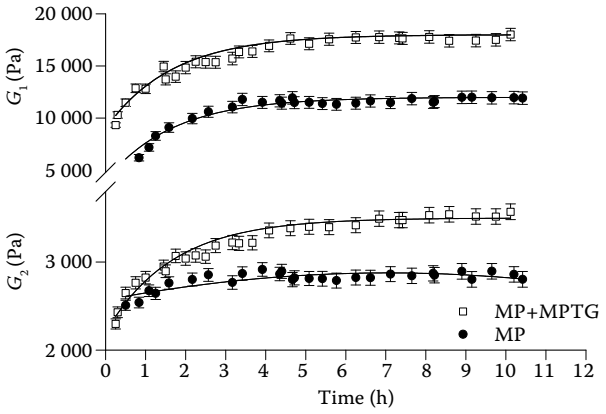
$$G(t) = G_0 + (G - G_0)\,(1 - e^{-k}) \quad (1)$$

where:

G_1, loss G_2 – initial and final equilibrium values of the elasticity and loss moduli

k – constant of the crosslinking kinetics which determines the velocity of the structuring process

The kinetics constant k obtained as a result of theoretical curves fitting in accordance with Eq. (1) to experimental values assumes higher values in the protein systems with the addition of transglutaminase (Table 2). The molecular mechanism responsible for the described phenomena is complex and can be attributed to the fact that the isolates of myofibrilar proteins obtained from MRPM are in fact a mixture of various proteins, primarily fibrillar, i.e. mainly of the actomyosin complex and sarcoplasmatic and globular proteins. Sarcoplasmatic and globular proteins are water-soluble in contrast to fibrillar proteins. Consequently, such a mixture can be treated as a dispersive system made up of two phases, i.e. hydrocolloidal continuous phase as well as dispersed phase made up of non-soluble myofibrilar proteins. This exerts a



MP – myofibrilar preparation; MRPM – mechanically recovered protein meat

Figure. 1. Kinetics of values changes of the dynamic elasticity modulus G_1 and the loss modulus G_2 during the incubation process of myofibrilar preparation (n = 5)

significant influence on the rheological properties of the examined system reflecting its composition. At a given protein concentration in the system as well as constant incubation temperature, the increase of elasticity of the examined systems in the time function occurs as a result of the expansion of bonds between protein macromolecules. This exerts a direct impact on the dynamics and differentiation of both the elasticity G_1 and loss G_2 moduli between the protein systems containing MPTG and the control (Figure 1).

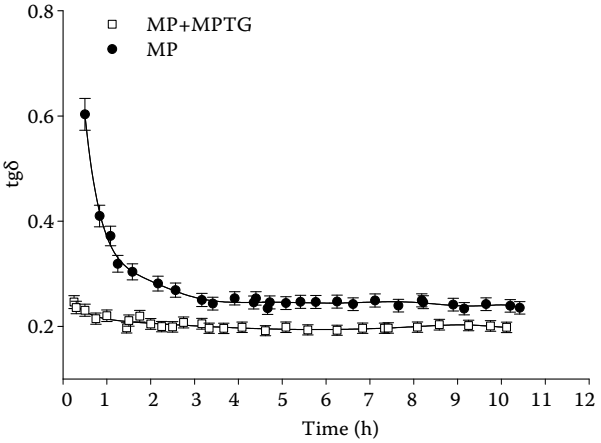
In the course of the entire analysed time interval, distinctly higher values could be observed of both the elasticity G_1 and loss G_2 moduli in the protein systems supplemented with MPTG in comparison with the control system. This is the result of the variability of molecular processes determining the values of these constituents. This is confirmed by the values of k (G_1) and k (G_2) constants (Table 2) determining the change dynamics of, respectively, the true G_1 and imaginary G_2 constituents of the combined rigidity modulus of the examined systems.

Appropriately to the time changes of the component values of rigidity moduli, the changes in the time courses of the lost tangent tgδ values were analysed (Figure 2). Tgδ values in the analysed incubation time interval in the fresh protein system containing MPTG were found to be at the lower level of values in relation to the control system indicating its smaller capability for energy dispersion. In the control system (MP), within 4- to 5-h time interval, the changes in tgδ values take place much more slowly as evidenced by maximum of losses. This can probably be caused by the occurring proteolysis of myofibrilar proteins leading to their fragmentation as well as an increase of their solubility. The impact of proteolytic protein transformations on the dynamics of the conformational changes is limited to a considerable extent by MPTG supplementation (Yongsawatdigul & Piyadhammaviboon 2004; Ionescu *et al*. 2008).

Table 2. Mean values and standard deviation of the crosslinking kinetics constant obtained as a result of fitting of theoretical curves in accordance with Eq. (1) to experimental values

Modulus	Type of sample	$k \pm S_d$	R^2
Elasticity G_1	MP	0.0114 ± 0.0005	0.96
	MP+MPTG	0.0120 ± 0.0006	0.93
Loss G_2	MP	0.0095 ± 0.0010	0.89
	MP+MPTG	0.0107 ± 0.0006	0.93

n = 5; ± standard deviation; MP – myofibrilar preparation; MRPM – mechanically recovered protein meat



MP – myofibrilar preparation; MRPM – mechanically recovered protein meat

Figure 2. Kinetics of value changes of the lost tangent tgδ during the incubation process of myofibrilar preparation (n = 5)

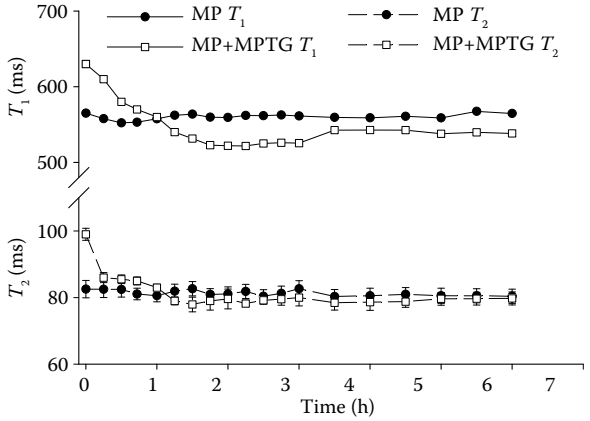
There is little information in the literature on the subject concerning rheological changes of unheated MP modified enzymatically. Certain information was published earlier by Hwang *et al*. (2007) but their data referred to the rheological characteristics of fish surimi subjected to the action of increased pressure.

NMR analysis

The values of spin-lattice relaxation times T_1 determined using the NMR technique depend on the relative proportions of free and bound water. The material containing more water is characterised by a longer relaxation time. Figure 3 presents the time changes in the values of spin-lattice T_1 and spin-spin T_2 relaxation times for the two analysed systems.

In the case of the control sample, slight changes were observed in T_1 relaxation times. The addition of MPTG increased significantly the values of the relaxation time during the initial phase of the experiment. Minimum T_1 values were observed after about 2–2.5 h of transglutaminase interaction with MP proteins and this parameter reached lower values than in the control sample. Hence, the obtained results indicate distinct water binding by MP proteins molecules.

The spin-spin relaxation process determined by the relaxation time T_2 reflects molecular dynamics of water molecules. In the analysed systems,



MP – myofibrilar preparation; MRPM – mechanically recovered protein meat

Figure 3. Time changes in values of spin-lattice T_1 and spin-spin T_2 relaxation times in the protein incubation process of myofibrilar preparation (n = 4)

T_2 values were several times lower in comparison with T_1 values. The analysis of the obtained results revealed that the examined systems were characterised by one T_1 and T_2 relaxation time confirming a considerable distribution uniformity of water molecules in the system. In the case of the preparation supplemented with MPTG, the water dynamics was considerably higher in comparison with that in the control sample at the moment of the experiment initiation (Figure 3) as manifested by higher values of spin-spin relaxation times. As such changes did not occur in the control sample, the changes in the spin-spin relaxation time were probably caused by the long-term influence of the enzyme on proteins which could be associated with biochemical transformations taking place in the studied system.

It is evident from the presented research results on the relaxation times that the most dynamic changes of water binding occurred during the first hours of the modification process. In this period, intensive water binding by proteins took place. In addition, the shortening of the T_2 time was connected with the inhibition of molecular water dynamics. Probably, water was bound in the sorption centres on the protein surface which became exposed as a result of enzyme activity. Biochemical changes in the examined system influenced the initiation of the loosening of water bound by protein molecules and, consequently, its re-liberation.

Water activity. The transport process of the diffusing substance in the medium is described by the following diffusion equation:

$$J = D_a \frac{da}{dx} \quad (2)$$

where:

J – density of the diffusion flux which is proportional to the activity gradient (da/dx)

D_a – activity diffusion coefficient determined on the basis of the measurements of kinetics activity which serves as a proportionality coefficient. The measurements of this kinetics also allow the determination of the equilibrium value of water activity a_{oc} in the sample

Table 3 presents the results of the determination of the equilibrium activity (a_{oc}) and diffusion coefficients (D_a) for the investigated systems obtained in the course of a measurement series which lasted over 7 hours.

Table 3. Mean values and standard deviation of the equilibrium activity and diffusion coefficients obtained for the control sample (MP) and for the sample of transglutaminase modified myofibrilar preparation (MP+MPTG)

Incubation time (h)	MP		MP+MPTG	
	a_{oc}	$D_a \times 10^{-10}$ (kg/m/s)	a_{oc}	$D_a \times 10^{-10}$ (kg/m/s)
1.5	0.908 ± 0.002	140 ± 12	0.925 ± 0.002	210 ± 15
2.3	0.907 ± 0.002	136 ± 10	0.929 ± 0.002	202 ± 13
3.2	0.908 ± 0.002	122 ± 11	0.932 ± 0.002	181 ± 14
4.0	0.908 ± 0.002	136 ± 14	0.931 ± 0.002	206 ± 13
4.9	0.913 ± 0.002	97 ± 9	0.935 ± 0.002	189 ± 16
5.8	0.914 ± 0.002	100 ± 12	0.932 ± 0.002	203 ± 16
6.6	0.913 ± 0.002	104 ± 13	0.930 ± 0.002	189 ± 14
7.4	0.913 ± 0.002	100 ± 10	0.934 ± 0.002	180 ± 13

n = 5; ± standard deviation; MP – myofibrilar preparation; MRPM – mechanically recovered protein meat

The changes in the equilibrium activity values and diffusion coefficients in a given sample are relatively small in the course of time and, to a considerable extent, accidental, and they remain within the boundary of the measurement errors of the system. Transglutaminase-modified systems are characterised by a higher value of the mean activity in comparison with the control system. Moreover, it was also found that the mean diffusion coefficient in the material of the modified sample assumed the value almost twice higher in comparison with the control system.

In the light of Flory (1953) thermodynamic theory, the increase of water equilibrium activity in enzymatically modified samples should be interpreted as the outcome of the development within the system of additional intermolecular bonds (Δn_j). It is also evident from the analysis of dependence that the gradual increase of the extent of crosslinking of the polymer system may lead to the achievement of equilibrium activity equal to the activity of the pure solvent. Further crosslinking may result in the effect of syneresis manifesting itself in the drip of the solvent outside the polymer system.

According to the reports of some researchers, the addition of enzymatic preparation decreased the volume of thermal drip from protein gels from red and poultry meat (Pietrasik *et al*. 2007). Presumably, the observed advantageous effect of the MTG addition may be attributed to the myofibril swelling and more efficient water binding as well as to an improved capability of muscle proteins to form gel networks (Ruiz-Carrascal & Regenstein 2002). However, other researchers reported a neutral or negative influence of the MTG addition in the presence of salts on the water binding capability in meat products (Kilic 2003; Carballo *et al*. 2006). This negative MTG impact on meat protein system was probably caused by a decreased capability of water binding by salt-soluble proteins attributable to the developed cross linking. A similar effect was observed in the case of excessive MTG addition as well as excessively high temperature of the crosslinking reaction (40°C) (Ruiz-Carrascal & Regenstein 2002).

CONCLUSIONS

The molecular mechanism responsible for the described phenomena is complex. It results from the fact that, in reality, the isolates of myofibrilar proteins obtained from MRPM apart from actomyosin complex also contain certain quantities of sarcoplasmatic proteins as well as connective tissue. This significantly impacts on the rheological properties of the investigated samples. The changes in rheological parameters of the raw preparation supplemented with MPTG were probably caused by alterations in the relative free and bound water contents in the structure as confirmed by the results obtained from the investigations employing the NMR technique. The modification time longer than 3 h caused increases of free water quantities in the system which may indicate that the protein-water bonds were displaced by the stronger action of crosslinking bonds of the protein-protein type. During a similar duration period of the modification, a dynamic process of protein crosslinking in the preparation took place determined on the basis of the elasticity modulus values. Moreover, the above remarks seem to be confirmed by the results obtained from the texture analysis. Simultaneously, the enzyme increased water molecule association by the MP proteins. This, in turn, caused greater dynamics of changes of the elasticity modulus values in the samples with MPTG in comparison with those without its addition.

The observed increase of equilibrium water activity in the modified samples should be interpreted as a result of additional inter-protein bonds development in the system. The performed analyses of the time changes of the relaxation time values as well as water equilibrium activity values revealed that the two parameters in the control sample failed to alter significantly over time. It was found that the enzymatic modification of the examined protein systems showed, in the time interval of approximately 4.5 h of incubation, an inverse correlation between the macroscopic parameters such as water activity and relaxation time, that is to say, the parameters determining the changes in water molecular dynamics at molecular level. This means that the analysis of the effect of the addition of enzyme to protein preparations requires research methods which could determine the changes at molecular level.

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